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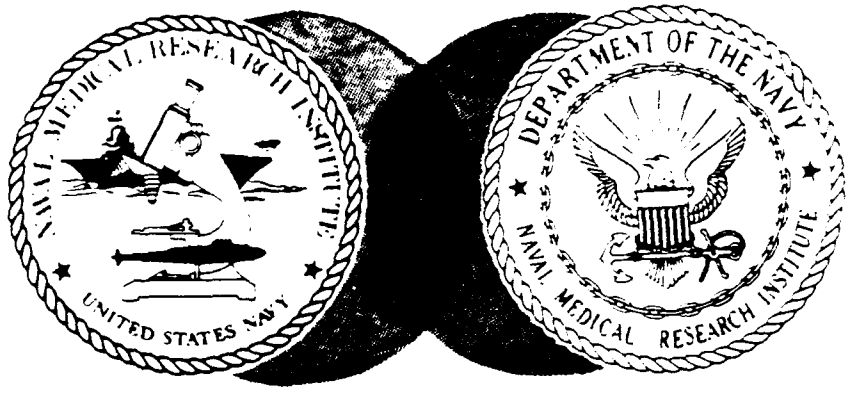

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PLASMA ATTENUATION OF ENDOTOXIN TOXICITY IN IRRADIATED AND TUMOR-BEARING MICE

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PLASMA ATTENUATION OF ENDOTOXIN TOXICITY IN IRRADIATED AND TUMOR-BEARING MICE

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R. I. WALKER, K. DEMARCO, G. D. LEDNEY and E. D. EXUM. Plasma attenuation of endotoxin toxicity in irradiated and tumor-bearing mice. *Toxicon* **19**, 857–861, 1981. We tested the hypothesis that citrated plasma from normal or irradiated mice and from normal rabbits can protect mice against endotoxin-induced lethality. In contrast to saline–endotoxin mixtures, challenge i.p. with 0.3 mg of *Salmonella typhi* endotoxin mixed with 1 ml of plasma from either normal or irradiated donors was not lethal for B6CBF1 mice irradiated 7 days previously with 1000 rads [^{60}Co]. In other experiments unirradiated mice were simultaneously inoculated i.p. with 0.8 mg of endotoxin and 1 ml of saline or normal rabbit plasma. This plasma also protected recipient animals challenged with endotoxin. Furthermore, rabbit plasma protected C57BL 6 mice whose sensitivity to 0.3 mg of endotoxin was enhanced due to implantation of the animals with a 3LL carcinoma 3 days previously. The evidence thus obtained confirms the hypothesis that plasma interferes with the lethal effects of the endotoxin.

INTRODUCTION

THE ENDOTOXIN component of the cell walls of gram-negative bacteria, when introduced into a susceptible animal, can elicit an inflammatory response that can be destructive, but may also elicit a reaction that is relatively benign and, in some cases, beneficial. For example, the injection of small amounts of endotoxin can increase resistance of an animal to a variety of micro-organisms (CAMPBELL and WHITE, 1976; WALKER *et al.*, 1976; TIZARD and RINGLIBERG, 1975), as well as induce regression of malignant tissue (CARSWELL *et al.*, 1975) and regeneration of damaged liver (ROSSOLINI *et al.*, 1976). The potential therapeutic benefits of endotoxin have led to numerous attempts to modify the structure of the compound so that beneficial effects are retained and detrimental aspects are eliminated (PRIGAL *et al.*, 1973; GALLEY *et al.*, 1975; SNYDER *et al.*, 1978). Recent studies of the host response to challenges with endotoxin have led to a better understanding of the normal process of endotoxin detoxification. Plasma components can modify or degrade endotoxin with reduction of toxicity (SKARNES and ROSEN, 1971; FUST and FORIS, 1974; JOHNSON *et al.*, 1977; ULEVITCH and JOHNSTON, 1978). These plasma–endotoxin interactions may be important to survival because plasma from endotoxin-resistant C3H/HeJ mice is rich in detoxifying substances (SULTZER and GOODMAN, 1977). Furthermore, dogs can be protected against death from endotoxin by prior administration of dog plasma containing adequate amounts of an unidentified heat-labile substance (WALKER *et al.*, 1980a). In this study we tested the hypothesis that plasma from normal mice and rabbits can be used to attenuate endotoxin *in vivo* in mice with altered resistance to the toxin.

MATERIALS AND METHODS

Animals

(C57BL × CBA) F1 Cum BR (henceforth designated B6CBF1) female mice and C57BL 6 BR female mice were obtained from Cumberland View Farms, Clinton, TN. They were housed for a period of 2 weeks in groups of 15 animals in a quarantine facility until a random sample was found to be free of histologic lesions of common murine diseases and until sterile water bottle cultures of all animals were free of *Pseudomonas*. The mice were 17–19 weeks old when used. At all times, the mice were kept on a 6 a.m. (light) to 6 p.m. (dark) cycle in filter-covered cages. Wayne Lab-Blox diet was provided throughout the quarantine and experimental time periods. Chlorinated (12 ppm) water was provided after the quarantine periods. Adult (3–4 kg) male New Zealand white rabbits obtained from local suppliers and maintained under a veterinarians supervision were used as plasma donors.

Radiation

Mice were placed in Plexiglas restrainers and exposed bilaterally to 1000 rads whole body gamma irradiation (45 rads/min) with opposing [^{60}Co] sources. This treatment causes death in 100% of the animals in approximately 14 days.

Tumor

The Lewis Lung (3LL) carcinoma was provided by the National Cancer Institute, National Institutes of Health, Bethesda, MD, in its 86th s.c. passage in male C57BL 6 mice. The tumor cells used in the experiments reported here were from the 111–113th passage maintained in male C57BL 6 mice at this institute. The methods for tumor cell preparations and s.c. injections were previously described (LEDNEY *et al.*, 1981).

Endotoxin

Salmonella typhi Lipopolysaccharide W (Difco, Detroit, Mich.) was used for all experiments. This endotoxin was suspended in sterile physiologic saline to a concentration of 1 mg/ml, placed into 10 ml vials and stored at −20°C until used.

Plasma

Rabbits and mice were anesthetized with metofane (methoxyflurane, Pitman-Moore Inc.). Rabbit blood was drawn from the heart through an 18 gauge hypodermic needle into a syringe with 15% acid citrate dextrose solution. Mouse blood was collected from the brachial artery. The blood was centrifuged at 1500 *g* for 10 min at room temperature to obtain clear plasma, which was then stored in 10 ml vials at −20°C until used. Prior to use, the plasma was thawed at room temperature and recentrifuged at 1500 *g* for 10 min at room temperature to remove cryoprecipitates. Platelet-rich plasma was obtained by centrifuging whole blood at 150 *g* for 10 min at room temperature and removing the supernatant from the cell pack.

RESULTS

Plasma endotoxin mixtures in irradiated mice

B6CBF1 mice were challenged i.p. with a freshly prepared mixture of 0.3 ml (0.3 mg) of endotoxin and 1 ml of saline 7 days after irradiation. Eighty-five percent of these animals died

TABLE 1. SURVIVAL OF MICE CHALLENGED WITH ENDOTOXIN AND TREATED WITH MOUSE OR RABBIT PLASMA

Experiment	Treatment*	No. of mice	% Mortality in 48 hr
1†	Saline	34	85
	Platelet-rich plasma from normal mice	28	21
	Plasma from normal mice	45	18
	Plasma from irradiated mice	20	20
2‡	Saline	14	64
	Plasma from normal rabbit	30	20

*Endotoxin and saline or plasma were mixed immediately prior to i.p. injection in Expt 1, but injected separately in Expt 2.

†Mice received 0.3 mg *Salmonella typhi* endotoxin 7 days after 1000 rads [^{60}Co] irradiation.

‡Unirradiated mice received 0.8 mg endotoxin.

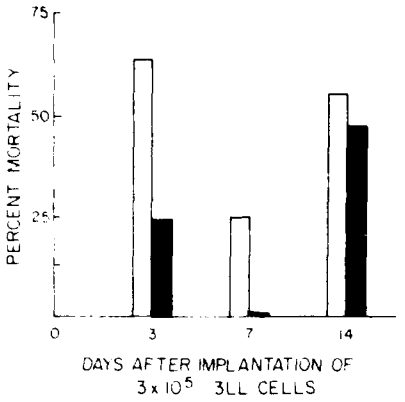

FIG. 1. COMPARISON OF MORTALITY IN TUMOR-BEARING MICE CHALLENGED WITH SALINE (CLEAR BARS) OR RABBIT PLASMA (DARK BARS) ENDOTOXIN PREPARATIONS ON DAYS 3, 7, OR 14 AFTER ENGRAFTMENT WITH TUMOR CELLS.
Each group contained 10 animals which were observed for 72 hr after challenge with endotoxin.

within 48 hr after challenge (Table 1). This mortality was reduced significantly when platelet-rich plasma from normal mice was substituted for saline. Comparable protection was also achieved with cell-free plasma obtained from normal or irradiated mice (Table 1).

Use of rabbit plasma to protect normal mice

Experiments were conducted to determine if plasma from rabbits, a more convenient blood donor, could be substituted for mouse plasma for further studies in mice (Table 1). Unirradiated B6CBF1 mice were challenged with 0.8 mg of endotoxin, followed immediately by treatment with 1 ml of saline or rabbit plasma. This procedure was used to prevent differences in time of preincubation of endotoxin and plasma prior to interaction with the host. The larger amount of toxin was used because the mice had not been irradiated. With this approach, 80% (24/30) of the mice treated with normal rabbit plasma survived the challenge with endotoxin, as compared to 36% (5/14) of the mice administered saline with the endotoxin challenge.

Plasma treatment of tumor bearing mice

C57BL/6 mice engrafted with tumor cells were inoculated with saline or rabbit plasma (1.3 ml) or 1 ml of saline or plasma and 0.3 ml (0.3 mg) of endotoxin. Mortality was determined over a 48 hr period. These challenges were administered to mice on days 3, 7 or 14 after transplantation with tumor cells. These days were selected because previous work (WALKER *et al.*, 1980b) demonstrated that increased sensitivity to challenge with 0.3 mg of endotoxin, for some unknown reason, occurred at days 3 and 14 after transplantation, but not at day 7. Treatment with plasma reduced the incidence of mortality, as compared to that noted for mice injected with saline and endotoxin at 3 days, but not at 14 days after transplantation (Fig. 1). Few deaths occurred in either saline- or plasma-treated mice challenged with endotoxin at 7 days after tumor engraftment.

DISCUSSION

Previously, DAS *et al.* (1974) showed that platelet-rich plasma protected rats against

endotoxin, but our data indicate that platelets were not necessary for the protection of mice. The nature of the protective factor(s) active in our study is unknown, but numerous such factors have been described (SKARNES and ROSEN, 1971; FUST and FORIS, 1974; UTEVITCH and JOHNSTON, 1978). Plasma may act on toxin structure, rather than host physiology, to increase resistance. This concept is consistent with the interspecies plasma protection observed. It is also noteworthy that both plasma and $ZnCl_2$ retard hepatosplenic uptake of endotoxin injected into the peritoneal cavity (WALKER *et al.*, 1978), but only plasma protects irradiated mice from endotoxin-induced lethality.

Plasma protection of animals made sensitive to endotoxin in two different ways (radiation and tumor) indicates that protection is probably not due to replacement of a factor lost in the sensitized animal. In fact, plasma from irradiated mice was just as useful as normal plasma in reducing mortality in recipient animals (Table 1).

Plasma treatment did not reduce mortality following endotoxin treatment at 14 days following engraftment. Since this treatment protected mice sensitized to endotoxin at other time intervals, it would seem likely that it is not the endotoxin *per se* that was responsible for mortality at 14 days. It has been reported that breakdown products of malignant tissue have toxic effects on the host (HAVAS *et al.*, 1960).

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